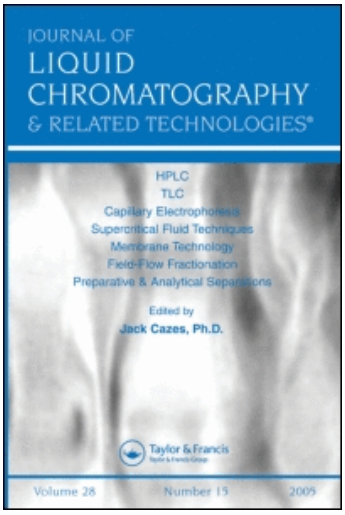

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STUDY OF THE LIPOPHILICITY OF ARYLPROPIONIC NON-STEROIDAL ANTI-INFLAMMATORY DRUGS. A COMPARISON BETWEEN LC RETENTION DATA ON A POLYMER-BASED COLUMN AND OCTANOL-WATER PARTITION COEFFICIENTS

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ABSTRACT

Molecular lipophilicity can be expressed by log P_{ow} or, more conveniently, by log k_w, i.e., determined by the traditional shake-flask technique, or by reversed-phase high performance liquid chromatography (RP-HPLC). Moreover, the unionized form of solutes is usually taken as the reference state for the measurement of lipophilicity. This can be problematic for the RP-HPLC determination of lipophilicity of acidic compounds due to the limited pH operating range of silica bonded phases.

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The measured dissociation constant values (pKa) of the twelve arylpropionic non steroidal antiinflammatory drugs (NSAIDs) are comprised between 3.80 and 5.70 ; consequently, the lipophilicities of the unionized forms must be measured at pH below 2. Accordingly, their capacity factors (log k_w) were determined on a column packed with a hydrophilic polymethacrylate gel having octadecyl groups. This RP-column allows separations at low pH values of the mobile phase. In practice, the values of log k_w are obtained by a series of isocratic measurements at various compositions of binary acetonitrile-water eluents and extrapolation of the relationship between log k and volume fraction of organic solvent, ϕ, to 100% water. The 1-octanol-water partition coefficients (log P_{ow}) of these NSAIDs were determined by the shake-flask technique using a conventional methodology.

A significant linear relationship was obtained between log P_{ow} and log k_w with a slope close to unity, indicating similar intrinsic thermodynamic behaviour of these drugs for the two partitioning processes.

This excellent correlation prompted us to validate this polymer-based column, to be useful for the determination of other acidic drug lipophilicity.

INTRODUCTION

The lipophilicities of drugs, commonly characterized by their 1-octanol-water partition coefficients (log P_{ow}), plays an important role in their pharmacological activity.[1] Although the choice of 1-octanol as a solvent reflecting the properties of the lipid components of the cell membrane has occasionally been questioned, the large number of 1-octanol-water partition data collected by Hansch and Leo[2] have made the partition system a common reference. To overcome several difficulties in making log P_{ow} measurements, several chromatographic approaches have been published which were summarized, in detail, by Braumann[3] and Kalisan.[4]

RP-HPLC still remains a method of choice, particularly when lipophilicity of highly lipophilic compounds (log $P_{ow} > 3$) has to be assessed. The alkylsilane-bonded phases, in particular octadecylsilane (ODS), are the most frequently used non-polar stationary phases to determine isocratic capacity factors (log k) which, when extrapolated to 100% water eluent, yield log k_w as a lipophilic index.

Some authors[5,6] emphasize that the chromatographic parameters (log k or log k_w), determined on ODS columns result as quite different from log P_{ow}, most likely due to strong polar interactions with free uncapped silanols. Besides, these phases are often unstable at a pH range wide enough to cover dissociation range

of acidic analytes. For this reason, we decided to use a polymer-based column packed with a polymethacrylate gel to measure the log k_w values of a series of twelve arylpropionic acids. Such a stationary phase has been documented to produce separations at lower or higher pH values of the mobile phase that cannot be performed with conventional C_{18} silica-based stationary phases.[6]

EXPERIMENTAL

Chemicals

The twelve NSAIDs studied are listed below. Carprofen, fenbufen, fenoprofen, indoprofen, ketoprofen, naproxen, pirprofen, and suprofen were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Alminoprofen (E. Bouchara, Levallois, France), flurbiprofen, and ibuprofen (Boots, Nottingham, UK) and tiaprofenic acid (Roussel Uclaf, Romainville, France) were generously supplied.

All chemicals and solvents were of analytical grade or HPLC grade. Water was obtained from a Milli-Q® purification system.

Apparatus and Chromatographic Conditions

HPLC was carried out at 22°C with a chromatograph equipped with a constant flow pump model *510* (Waters Assoc., Milord, MA, USA), an autosampler WISP model *717+* (Waters), a variable wavelength spectrophotometer model UV 150 (Thermo*Q*uest, Austin TX, USA) operating between 225 nm and 290 nm. The detection was performed at the maximum wavelength for each compound.

The compounds were chromatographied on a Shodex® RSpak D18-316 column (Showa Denko K.K., Shodex Separation & HPLC group) (150 mm x 6 mm I.D.) at a flow rate of 1 mL/min. This column is packed with hydrophilic polymethacrylate gel having octadecyl groups attached. The various mobile phase compositions ranged from 25% to 75 % acetonitrile with 0.2N KCl/HCl buffer (pH 1) (v/v). Chromatographic data were recorded on an integrator *Data Jet* (Thermo*Q*uest).

Determination of Capacity Factor (k')

All stock solutions contained 1mg/mL of each NSAID. They were prepared in methanol and subsequently diluted with water to the final injected concentrations (50 µL at 100 µg/mL). According to their chromatographic behaviour, the

retention time (tr) of each compound was determined in triplicate at six different acetonitrile-buffer mobile phase mixtures.

At each mobile phase composition, the capacity factor was calculated through the formula: $k' = (t_r - t_o) / t_o$, where t_o is the column dead-time of the system and was measured as the time from the injection to the first distortion of the baseline after drug injection. The log k values at 100% aqueous mobile phase (log k_w) were obtained from the y-intercept of plots of log k versus percent of organic modifier in the eluent.[7]

Measurement of Dissociation Constants (pKa)

For each compound, the pKa determinations were determined using a classical potentiometric method described elsewere.[8]

Determination of log P_{ow} of the Arylpropionic Acids by Shake-Flask

The 1-octanol-water partition coefficients of arylpropionic acids were determined by the shake-flask technique using a conventional methodology.[9] Briefly, samples in a weight range of 0.2-1 mg were partitioned between 2 mL of 1-octanol saturated with water at pH 1 and 20 mL of water at pH 1 saturated with 1-octanol. For alminoprofen, suspected to have log P_{ow} value of less than unity, a ratio of 10 mL of 1-octanol to 10 mL of water was used.

The resulting two-phase mixture was gently mixed for one hour on a slow rotative mixer (approx. 1 revolution/10s). After mixing, samples were centrifugated for 30 min. (2000 rpm) to ensure any possible emulsions were removed.[10] The water phase absorbance was measured at the maximum wavelength of each NSAID before, and just after, the partitioning experiment. Five independent measurements were performed for each compound, leading to a mean log P_{ow} value.

Determination of the Calculated Incremental Clog P Values

For the twelve arylpropionic acids, the Clog P values were calculated by a fragmental method available in MacLogP.[11]

RESULTS AND DISCUSSION

The aim of this study was to compare the partition coefficients of twelve arylpropionic acids, measured by the traditional shake-flake technique and by

HPLC, using a polymer-based column. Generally, for acids, log k_w was corrected for ionization in order to obtain apparent log k_w for neutral forms. Considering that the pKa values of these compounds are between 3.80 (tiaprofenic acid) and 5.70 (fenoprofen), their degrees of ionization (α) are negligible at pH 1 (Table 1). Consequently, we measured the lipophilicities of the unionized forms at pH 1.

Determination of Lipophilicity with the Polymer-Based Column

For all compounds, the capacity factors (log k) increased while the acetonitrile concentration in the eluent decreased when using our polymer-based column. Classically, for estimating lipophilicity by RP-HPLC, methanol is considered as the most suitable organic solvent.[3] With a polymer-based reversed-phase packing material, the manufacturer recommended the use of acetonitrile instead of methanol to obtain sharp peak shapes and shorter retention times.

In this study, we have chosen to measure the log k value extrapolated to 0% of the organic modifier in the mobile phase (log k_w). The log k_w is a standardized retention parameter which is more reliable than any arbitrarily selected isocratic log k.[12] For all arylpropionic acids, linear relationships ($r \geq 0.98$) were proven to exist between the log k values and acetonitrile concentrations (ϕ), allowing the calculation of log k_w and S through extrapolation (Table 2 ; Equation 1):

$$\log k = a_0 + a_1\phi, \text{ or } \log k = \log k_w - S\phi \quad (1)$$

Table 1. pKa Values and Ionization Percentages (α) at pH 1 for the Arylpropionic Acids

Compounds	pKa	α (%)
Alminoprofen	5.02	0.01
Carprofen	4.36	0.004
Fenbufen	5.60	0.002
Fenoprofen	5.70	0.03
Flurbiprofen	4.20	0.06
Ibuprofen	4.55	0.03
Indoprofen	4.25	0.05
Kétoprofen	4.18	0.08
Naproxen	4.20	0.01
Pirprofen	4.64	0.02
Suprofen	4.11	0.07
Tiaprofenic acid	3.80	0.16

α calculated as : $\alpha = 1/(1+\text{antilog}(\text{pKa} - \text{pH}))$.

Table 2. Linear Correlations log k = a_0 + $a_1\phi$, Obtained by HPLC for the Twelve Arylpropionic Acids

Compounds	log k_w (a_0)	−S (a_1)	r	s_{a0}	s_{a1}	s
Alminoprofen	1.034	−0.031	0.996	0.026	0.0007	0.024
Carprofen	4.975	−0.054	0.993	0.120	0.0016	0.029
Fenbufen	3.718	−0.043	0.993	0.086	0.0012	0.027
Fenoprofen	3.946	−0.047	0.993	0.101	0.0014	0.027
Flurbiprofen	4.352	−0.051	0.993	0.098	0.0010	0.031
Ibuprofen	4.130	−0.051	0.993	0.099	0.0013	0.031
Indoprofen	3.039	−0.039	0.999	0.028	0.0004	0.009
Ketoprofen	2.990	−0.038	0.999	0.018	0.0002	0.004
Naproxen	3.637	−0.043	0.994	0.079	0.0011	0.025
Pirprofen	2.022	−0.024	0.980	0.075	0.0010	0.030
Suprofen	3.005	−0.038	0.997	0.045	0.0006	0.015
Tiaprofenic acid	3.339	−0.042	0.994	0.099	0.0010	0.031

s_{a0}, s_{a1} : standard errors for the intercept a_0 and for the slope a_1, respectively.
s : fit standard error.
r : correlation coefficient.

The slopes, S, for the equations, were mostly constant; this is related to the structural similarities of the molecules. This slope a_1 (-S) is negative in all cases, and it is supposed to be related to the hydrophobic surface of the molecule which interacts with the non-polar stationary phase.[13]

Determination of 1-Octanol-Water Partition Coefficients

Table 3 groups the log P_{ow} values (mean ± SD ; n=5) corresponding to the twelve NSAIDs. The lower and upper values were 0.618 (for aminoprofen) and 4.128 (for carprofen), respectively.

It is well known that many factors can affect the measurement of partition coefficient; among them are temperature, lack of mutual phase saturation, solute and solvent purity, and time to reach equilibrium. Consequently, there are considerable variations among published log P_{ow} values. Using the experimental conditions recommended by Dearden et al.,[10] our measured 1-octanol-water partition coefficients agree with those reported in the literature (log P_{lit} ; Table 3).

Table 3. log P Values for Arylpropionic Acids

Compounds	log P_{ow} (Mean ± SD)	log P_{lit}	Clog P
Alminoprofen	0.618 ± 0.002		2.19
Carprofen	4.128 ± 0.214		3.98
Fenbufen	3.138 ± 0.048	3.39 [a]	3.11
Fenoprofen	3.449 ± 0.112		3.82
Flurbiprofen	3.769 ± 0.087	4.16 [b]; 3.99 [c]	3.75
Ibuprofen	3.686 ± 0.105	3.50 [b]	3.50
Indoprofen	2.391 ± 0.100	2.77 [b]	2.74
Ketoprofen	2.683 ± 0.142	3.12 [b]	2.76
Naproxen	2.998 ± 0.126	3.18 [a]; 3.34 [b]	2.82
Pirprofen	1.765 ± 0.100		2.55
Suprofen	2.659 ± 0.051		2.54
Tiaprofenic acid	2.858 ± 0.032		2.54

[a] Data taken from ref. 14.
[b] Data taken from ref. 15.
[c] Data taken from ref. 16.

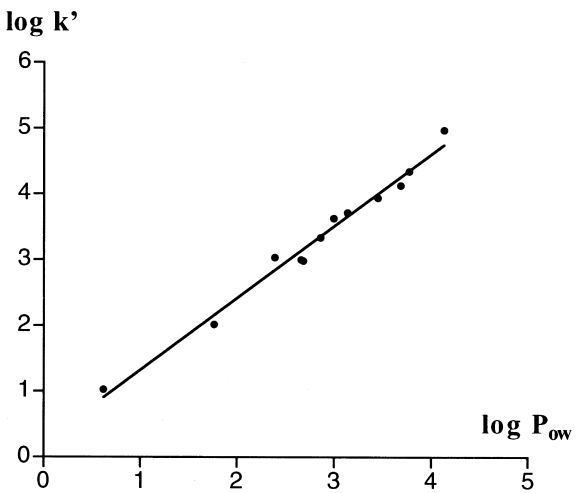

Figure 1. Plot of log k' versus log P_{ow}.

Correlation Between Lipophilic Indexes

The log k_w values were correlated with log P_{ow}, according Equation 2 for the experimental data listed in Tables 2 and 3. Figure 1 shows the correlation between the two methods.

$$\log k_w = 1.095\ (\pm\ 0.046)\ \log P_{ow} + 0.232\ (\pm\ 0.137) \qquad (2)$$
$$(n = 12,\ r = 0.991,\ s = 0.146,\ F = 564.9\ ;\ p < 0.0001)\ ;$$

where n is the number of data points, r is the correlation coefficient, s the standard error estimate, F is a measure of the significance of the correlation, and p is the probability level.

This satisfactory linear correlation with a slope close to unity indicates similar intrinsic thermodynamic behaviour of these NSAIDs for the two partitioning processes.[17] The described RP-HPLC system mimics partitioning in 1-octanol-water and, consequently, it is a valid model for the measurement of lipophilicities of these compounds in their unionized forms.

Another correlation can be calculated between log P_{ow} and the calculated log P values (Clog P) (Table 3 ; Equation 3).

$$\text{ClogP} = 0.535\ (\pm\ 0.099)\ \log P_{ow} + 1.501\ (\pm\ 0.297) \qquad (3)$$
$$(n = 12,\ r = 0.862,\ s = 0.316,\ F = 28.98,\ p = 0.0003)$$

The linear correlation between Clog P *vs* log P_{ow} is less significant than the relationship between log k_w *vs* log P_{ow} (Equation 2). It is well recognized that the calculated log P values (ClogP) are a standard method which is useful to give a first indication of molecular lipophilicity. This fragment constant approach estimates log P with sufficient accuracy for a wide range of structures. But sometimes, the calculation of lipophilicity is incomplete due to "missing fragments."[18]

CONCLUSION

Reversed-phase HPLC with C_{18} silica is the conventional support for lipophilicity determination. Our decision to use a polymer-based column for log k_w measurement was based upon a preference to have a packing material which would be resistant at low pH to estimate the lipophilic character of the unionized forms of these NSAIDs. Though any given log k value is specific to one HPLC system, the chosen LC column should mimic partitioning in 1-octanol-water in terms of hydrophobic and hydrophilic interactions between the stationary and mobile phases. This polymer-based reversed-phase adsorbent is comprised of a

hydrophilic polar polymeric support (polymethacrylate gel) covered with a hydrophobic surface-layer (C_{18} groups), and it has similar retention properties to the C_{18} silica packing.[19, 20] Therefore, this polymer-based column appears to be suitable for measuring the lipophilic character of unionized forms of drugs with a strong acidic behaviour.

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